Kinetics of infectivity are dissociated from PrP accumulation in salivary glands of Creutzfeldt-Jakob disease agent-inoculated mice

Suehiro Sakaguchi,1 Shigeru Katamine,1 Kouichi Yamanouchi,1 Masao Kishikawa,2 Ryozo Moriuchi,1 Norio Yasukawa,1 Takashi Doi1 and Tsutomu Miyamoto1*

1 Department of Bacteriology and 2 Department of Pathology, Scientific Data Center for the Atomic Bomb Disaster, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan

The protease-resistant isoform of prion protein (PrP) has been implicated in the pathogenesis and transmission of Creutzfeldt-Jakob disease (CJD), scrapie and other related diseases, but the relationship between the infectious agent and PrP awaits elucidation. In the present study, we have examined levels of infectivity together with accumulation of the protease-resistant form of PrP (PrP\textsuperscript{CJD}) in various tissues of CJD agent-inoculated mice. Accumulation of PrP\textsuperscript{CJD} occurred only in tissues, including brain, salivary gland and spleen, in which infectivity was readily detectable throughout the course of the experiment. The brain showed the highest levels of both infectivity and PrP\textsuperscript{CJD} accumulation, with well correlated kinetics. On the other hand, the high titres of infectivity detected in salivary gland and spleen early after inoculation of the agent were obviously distinguishable from PrP\textsuperscript{CJD}. Furthermore, in the salivary gland, the kinetics of infectivity and the accumulation of PrP\textsuperscript{CJD} reversed; infectivity declined as PrP\textsuperscript{CJD} accumulated in the tissue. Our findings indicate that PrP\textsuperscript{CJD} accumulation is associated with replication of the agent; however, PrP\textsuperscript{CJD} is unlikely to be the agent itself.

Introduction

Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler syndrome and kuru in man, and scrapie in animals are neurodegenerative diseases transmissible to laboratory animals (Chandler, 1961; Gajdusek et al., 1966; Gibbs et al., 1968). These diseases show characteristic pathological changes in the central nervous system, including vacuolation of neurons, astrogliosis and the development of amyloid plaques (Zlotnik & Rennie, 1965; Marsh & Kimberlin, 1975; Bendheim et al., 1984). The amyloid fibrils accumulated in the involved brain tissue consist of an aggregated, partly protease-resistant form of prion protein, PrP\textsuperscript{CJD} in CJD and PrP\textsuperscript{SE} in scrapie (Prusiner et al., 1983). PrP\textsuperscript{CJD} (PrP\textsuperscript{SE}) is derived from cellular PrP, PrP\textsuperscript{SE}, by a post-translational process (Chesebro et al., 1985; Oesch et al., 1985). Several lines of evidence have implicated PrP\textsuperscript{CJD} (PrP\textsuperscript{SE}) in transmission as well as pathogenesis of the diseases (Prusiner, 1982, 1991; Kitamoto et al., 1989; Hsiao et al., 1990; Prusiner & DeArmond, 1990; Prusiner et al., 1990). Infectivity and PrP\textsuperscript{SE} always copurify from the brain tissue of scrapie-infected hamsters in different purification procedures (Prusiner et al., 1983; Takahashi et al., 1986). Furthermore, the susceptibility of a host to scrapie is influenced by the nature of its allele of a single-copy gene encoding PrP (Prusiner et al., 1990). It has been proposed that PrP\textsuperscript{CJD} (PrP\textsuperscript{SE}), once introduced into normal cells, converts cellular PrP\textsuperscript{SE} into its protease-resistant form by an unknown mechanism (Prusiner & DeArmond, 1990; Prusiner, 1991; Weissmann, 1991). However, Czub et al. (1986, 1988) and Rubenstein et al. (1991) previously demonstrated that the accumulation of PrP\textsuperscript{SE} occurred well after the increase in infectivity in the tissues of scrapie agent-inoculated animals. Furthermore, Xi et al. (1992) recently described a more obvious dissociation between infectivity and PrP\textsuperscript{SE} accumulation in the brains of mice treated with amphotericin B. These findings oppose the identity of PrP\textsuperscript{SE} as the infectious agent. The presence of an undetected, putative nucleic acid in the infectious agent has also been hypothesized. In the virino hypothesis, the infectious agent consists of a nucleic acid genome and a coat protein, PrP (Dickinson & Outram, 1988). Weissmann (1991) recently proposed another hypothesis, the unified theory, in which PrP\textsuperscript{SE} (PrP\textsuperscript{CJD}) is associated with a putative nucleic acid; PrP\textsuperscript{SE} itself can cause transmission of the disease, while the nucleic acid is modulating the biochemical properties of PrP\textsuperscript{SE}. The nature of the infectious agent, particularly its relation to PrP, is still controversial (Manuelidis et al., 1987).

Although the pathological features characteristic for CJD and scrapie are restricted to brain tissue (Prusiner et al., 1990), replication of the infectious agent has been detected in other tissues such as spleen, lymph node,
salivary gland, intestine and skeletal muscle as well as in the brain (Chandler, 1961; Zlotnik & Rennie, 1965; Gajdusek et al., 1966; Eklund et al., 1967; Gibbs et al., 1968; Tateishi et al., 1979; Kimberlin & Walker, 1989). Interestingly, the agent replicates in the lymphoreticular system, including spleen tissue, long before the involvement of the brain (Eklund et al., 1967; Kuroda et al., 1983). Eklund et al. (1967) also demonstrated that the salivary gland gave a high level of infectivity much earlier than the brain of mice inoculated with the scrapie agent. In contrast to the brain, the infectivity initially detected in spleen and salivary gland persisted but did not increase thereafter. This suggested the possibility that the mode of agent replication in these tissues is distinct from that in the brain. In the present study, to elucidate the relationship between the infectious agent and PrP<sub>CJD</sub>, we examined the kinetics of infectivity together with the level of PrP<sub>CJD</sub> in various tissues, including spleen and salivary gland, of CJD agent-inoculated mice.

Methods

Animals. Specific pathogen-free male ddY mice (Japan SLC) aged 4 weeks were used in all experiments on CJD agent transmission. Animal experiments were conducted in the biohazard prevention area (P3) of the Laboratory Animal Center for Biomedical Research of the authors’ school and under the Guidelines for Animal Experimentation, Nagasaki University.

CJD agent. The Fukuoka 1 strain (Tateishi et al., 1979), kindly provided by Dr J. Tateishi, was passaged twice in the brains of ddY mice. Inoculated mice were sacrificed just after the onset of CJD and brain tissues were removed. Brains of 15 CJD mice were pooled and homogenized in PBS. The homogenate, adjusted to 20% (w/w) with PBS, was used as the CJD agent in the following experiments.

Determination of LD<sub>50</sub> of the CJD agent. The CJD agent was serially diluted 10-fold with PBS, ranging from 10<sup>0</sup> to 10<sup>-5</sup>, and 0.02 ml per head of each dilution was inoculated intracerebrally into five or six mice. The inoculated mice were observed until 364 days after inoculation and the incubation period was determined as described above. The diagnosis of CJD was confirmed by pathological changes in the brains of all dead or sacrificed mice. The titre of infectivity was calculated from the mean value of incubation periods on the basis of a standard curve between titre and incubation period.

Purification of PrP<sub>CJD</sub>. Frozen tissues used for infectivity titration were homogenized in 10 mm-sodium phosphate buffer pH 7.4, containing 10% sarcosyl. The homogenate was incubated with 150 µg/ml of DNase I at 37 °C for 60 min and centrifuged at 22000 g for 30 min. The supernatant was centrifuged at 215000 g for 120 min. The resulting pellet was sonicated in 1% sarcosyl buffer (10 mm-sodium phosphate pH 7.4, 1% sarcosyl and 10% NaCl) and centrifuged at 305000 g for 60 min. The pellet was treated with 25 µg/ml of proteinase K at 37 °C for 60 min and centrifuged at 150000 g for 15 min. The final pellet was used as a purified fraction of PrP<sub>CJD</sub>.

Immunoblotting. The purified PrP<sub>CJD</sub> was separated by SDS-PAGE under reduced conditions (Laemmli, 1970) and transferred electrophotographically to a nitrocellulose membrane (Towbin et al., 1979). The blot was blocked in PBST (PBS with 0.2% Tween 20) containing 5% non-fat dry milk. After washing with PBST, the blot was incubated with a rabbit antisera raised against synthetic peptides corresponding to the amino-terminal region of hamster PrP (a kind gift of Dr M. Shinagawa) at a dilution of 1:2000 in PBST containing 0.2% BSA (Shinagawa et al., 1986). The resulting immune complex was visualized by autoradiography after labelling with 125I-Protein A (Amersham). The radioactivity of bound 125I was quantified using the Bio-Image Analyzer (BAS 2000, Fuji Film) and the relative quantity of PrP<sub>CJD</sub> was estimated as the radioactivity (c.p.m.) per gram of tissue.

Results

Relationship between infectivity titre and incubation period

Male ddY mice aged 4 weeks were inoculated with 0.02 ml per head of serial 10-fold dilutions of the CJD agent. Mortalities, incubation periods and survival periods of the inoculated mice are shown in Table 1. According to the method of Kärber (1931), the LD<sub>50</sub> of the agent was calculated as 10<sup>-9</sup> per gram of tissue. Fig. 1 shows the correlation between the incubation period and the dilution of the agent. A linear relationship was obtained in the range of incubation periods between 108.5 and 192.5 days. Since the LD<sub>50</sub> of the agent used was 10<sup>-9</sup>/g, the relationship between the infectivity titre and the incubation period is given by

log<sub>10</sub>(LD<sub>50</sub>/g) = 13.067 - 0.0476y, 108.5 < y < 192.5. In this formula, y represents the incubation period, in days.

Infectivities in various tissues of CJD agent-inoculated mice

Mice were inoculated with the CJD agent, 10<sup>-8</sup> LD<sub>50</sub> units per head, intracerebrally. Pathological changes in the brain tissue and clinical signs of CJD became obvious approximately 7 and 14 weeks after inoculation, respectively. The inoculated mice were sacrificed 6 h (0 week) post-inoculation (p.i.) and then every 2 weeks until 14 weeks, and brain, salivary gland, spleen, liver and kidney tissues were removed. Infectivity of each
tissue was examined by inoculating the tissue homogenate into four to six mice. The titre of infectivity was calculated from the mean incubation period of the inoculated mice according to the formula described above. As shown in Table 2, infectivity was detected in all the tissues examined at 6 h p.i. (indicated as week 0 in the table). The infectivities of liver and kidney declined to undetectable levels by 2 weeks and were barely detected thereafter. In brain, the titre of infectivity declined a little, from $10^{5.50}$ to $10^{4.97} \text{LD}_{50}/g$, during the first 4 weeks, and the logarithmic increase in titre occurred 6 weeks p.i., reaching a level of more than $10^{7.90} \text{LD}_{50}/g$ at 14 weeks. Although infectivities of salivary gland and spleen also continued to be readily detectable until 14 weeks, kinetics of the titre in these tissues appeared to be distinct from those in the brain. The titre of the salivary glands rose nearly 100-fold, from $10^{6.21}$ to $10^{6.83} \text{LD}_{50}/g$, during the first 2 weeks and the maximum titre, $10^{6.83} \text{LD}_{50}/g$, was reached by 4 weeks. This level of titre was maintained until 8 weeks, then declined gradually reaching the lowest, $10^{6.02} \text{LD}_{50}/g$, by 14 weeks. The spleen tissue also showed a rapid increase in infectivity soon after inoculation of the agent, reaching a titre of $10^{6.07} \text{LD}_{50}/g$ by 8 weeks, and then fluctuating between $10^{6.07}$ and $10^{6.55} \text{LD}_{50}/g$.

Table 2. Infectivities of tissues of mice inoculated with CJD agent at various intervals after inoculation

<table>
<thead>
<tr>
<th>Time p.i. (weeks)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality*</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>4/4</td>
<td>6/6</td>
<td>6/6</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Incubation period</td>
<td>138 ± 14</td>
<td>146 ± 12</td>
<td>149 ± 12</td>
<td>122 ± 13</td>
<td>115 ± 13</td>
<td>110 ± 8</td>
<td>110 ± 11</td>
<td>95 ± 8</td>
</tr>
<tr>
<td>Estimated titre</td>
<td>5.50</td>
<td>5.12</td>
<td>4.97</td>
<td>6.26</td>
<td>6.59</td>
<td>6.83</td>
<td>6.83</td>
<td>&gt; 7.90</td>
</tr>
<tr>
<td><strong>Salivary gland</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality*</td>
<td>5/5</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>4/4</td>
</tr>
<tr>
<td>Incubation period</td>
<td>163 ± 13</td>
<td>121 ± 8</td>
<td>121 ± 8</td>
<td>125 ± 6</td>
<td>126 ± 17</td>
<td>145 ± 11</td>
<td>136 ± 10</td>
<td>150 ± 12</td>
</tr>
<tr>
<td>Estimated titre</td>
<td>4.31</td>
<td>6.31</td>
<td>6.31</td>
<td>6.12</td>
<td>6.07</td>
<td>5.17</td>
<td>5.59</td>
<td>4.93</td>
</tr>
<tr>
<td><strong>Spleen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality*</td>
<td>5/5</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Incubation period</td>
<td>170 ± 13</td>
<td>137 ± 6</td>
<td>126 ± 5</td>
<td>127 ± 7</td>
<td>116 ± 10</td>
<td>136 ± 9</td>
<td>126 ± 12</td>
<td>116 ± 7</td>
</tr>
<tr>
<td>Estimated titre</td>
<td>3.98</td>
<td>5.55</td>
<td>6.07</td>
<td>6.02</td>
<td>5.59</td>
<td>5.97</td>
<td>6.55</td>
<td>6.55</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality*</td>
<td>6/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Incubation period</td>
<td>162 ± 21</td>
<td>255 ± 55</td>
<td>203 ± 8</td>
<td>283 ± 91</td>
<td>425 ± 40</td>
<td>243 ± 19</td>
<td>430 ± 49</td>
<td>270 ± 98</td>
</tr>
<tr>
<td>Estimated titre</td>
<td>4.66</td>
<td>&lt; 2.90</td>
<td>&lt; 2.90</td>
<td>&lt; 2.90</td>
<td>&lt; 2.90</td>
<td>&lt; 2.90</td>
<td>&lt; 2.90</td>
<td>&lt; 2.90</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality*</td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Incubation period</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
<td>250 ± 7</td>
</tr>
</tbody>
</table>

* Dead mice/total mice inoculated.
† Mean no. of days ± S.D.
‡ $\log_{10}(\text{LD}_{50} \text{ unit/g tissue})$. 

---

**Table 1. Mortalities, survival periods and incubation periods of mice inoculated with serial 10-fold dilutions of the CJD agent**

<table>
<thead>
<tr>
<th>Dilution of agent (log_{10} dilution)</th>
<th>Mortality (dead/total)</th>
<th>Survival period (mean no. of days ± S.D.)</th>
<th>Incubation period (mean no. of days ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5/5</td>
<td>124.8 ± 12.7</td>
<td>103.8 ± 12.4</td>
</tr>
<tr>
<td>-1</td>
<td>6/6</td>
<td>145.8 ± 7.1</td>
<td>108.5 ± 12.3</td>
</tr>
<tr>
<td>-2</td>
<td>6/6</td>
<td>155.2 ± 8.7</td>
<td>128.5 ± 5.7</td>
</tr>
<tr>
<td>-3</td>
<td>6/6</td>
<td>173.2 ± 7.0</td>
<td>147.0 ± 7.7</td>
</tr>
<tr>
<td>-4</td>
<td>6/6</td>
<td>201.3 ± 34.3</td>
<td>172.7 ± 39.3</td>
</tr>
<tr>
<td>-5</td>
<td>4/5</td>
<td>205.0 ± 30.0</td>
<td>192.5 ± 31.0</td>
</tr>
<tr>
<td>-6</td>
<td>1/5</td>
<td>209</td>
<td>182</td>
</tr>
<tr>
<td>-7</td>
<td>0/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-8</td>
<td>0/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-9</td>
<td>0/6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Male ddY mice aged 4 weeks were inoculated with 0.02 ml of each dilution and observed every day until 364 days p.i. Onset of disease was determined as described in the text.

---

**Fig. 1. Correlation of incubation periods of CJD agent-inoculated mice with titres of the agent.** The CJD agent, $10^{7.9} \text{LD}_{50}/g$, was serially diluted 10-fold from $10^{0}$ to $10^{-5}$ and 0.02 ml of each dilution was inoculated into four to six mice intracerebrally. Time of incubation (mean number of days) of mice inoculated with each dilution was plotted.
Accumulation of PrP\textsubscript{CJD} in the tissues

The PrP\textsubscript{CJD} in some of the tissue homogenates used in the infectivity assay was purified and detected using immunoblotting. Purified fractions of PrP\textsubscript{CJD} were treated with proteinase K before immunoblotting to distinguish the normal cellular isoform, PrP\textsubscript{c}. Fig. 2(c) shows results only up to 14 weeks, but PrP\textsubscript{CJD} was detectable neither in kidney nor in liver throughout the course of the experiments. Although PrP\textsubscript{CJD} was not detected during...
the early time-course, it became detectable in brain, salivary gland and spleen 6, 4 and 6 weeks p.i., respectively (Fig. 2a to c). Maximal levels were reached by 14 weeks in all tissues, and rates of accumulation appeared to be similar. Three species of PrPCJD co-migrating with 26K, 22K and 19K markers, respectively, were detected in brain and salivary gland. In spleen, an additional fast-migrating band corresponding to 17K was observed.

Comparison of kinetics of infectivity titre and PrPCJD accumulation

Kinetics of PrPCJD accumulation in brain, salivary gland and spleen were analysed quantitatively and compared with those of infectivity (Fig. 3). The radioactivity of 125I-Protein A bound to PrPCJD on the immunoblots shown in Fig. 2 was determined and relative quantities of PrPCJD were expressed as radioactivities (c.p.m.) per gram of the tissue. A linear relationship of the radioactivity with the quantity of PrPCJD was obtained in the range from 1 to 500 mg of the 14 week brain tissue (data not shown). In brain, both time-course and rate of PrPCJD accumulation were fairly well correlated with those of increase in infectivity (Fig. 3a). On the other hand, as shown in Fig. 3(b, c), an obvious discrepancy between PrPCJD and infectivity was observed in the salivary gland, in particular, and spleen. During the early time after inoculation of the agent, both tissues showed high titres of infectivity in the order of 10^6 LD50/g, although PrPCJD was not yet detectable. Furthermore, between 8 and 14 weeks, the kinetics of infectivity and PrPCJD accumulation in salivary gland reversed. As a consequence, salivary gland at 14 weeks showed the highest level of PrPCJD accumulation despite its having the lowest value for the titre of infectivity.

Discussion

In the present study, we analysed kinetics of infectivity in various tissues of CJD agent-inoculated mice as compared with PrPCJD accumulation in tissues. Replication of the infectious agent first appeared in salivary gland and spleen soon after inoculation. In brain, the increase in infectivity was delayed and became obvious at 6 weeks. This confirms previous findings (Eklund et al., 1967; Kuroda et al., 1983) that the primary sites of agent replication are the salivary gland and spleen rather than the brain. No significant level of infectivity was detectable in kidney and liver. Among the tissues examined, PrPCJD accumulation was detected only in those which showed replication of the agent. Furthermore, the brain revealed a good correlation between infectivity and PrPCJD accumulation. These together indicate close association of PrPCJD with the infectious agent.

On the other hand, the high titres of infectivity in salivary gland and spleen early after inoculation were distinguishable from PrPCJD. Rubenstein et al. (1991) similarly showed that infectivity reached a maximum level in the spleen of scrapie-inoculated animals well before PrPCJD became detectable. Dissociation of infectivity from PrPCJD in the brain was also recently demonstrated by use of mice treated with amphotericin B. Both the clinical symptoms and the PrPCJD accumulation in scrapie-infected mice were retarded, and replication of the infectious agent was unaffected (Xi et al., 1992). This dissociation may indicate that PrPCJD (PrPCJD) is not essential for replication of the infectious agent, although the possibility remains that an extremely small, undetectable amount of the protein is sufficient to reveal infectivity. Recently, Büeler et al. (1992) reported the development of mice lacking a functional PrP gene. Surprisingly, the development and behaviour of these PrP-negative mice were normal. Thus, testing the susceptibility of the PrP-negative mice to scrapie or CJD agents may clarify whether PrP is an essential component of the infectious agent. The most important finding of the present study is that the kinetics of infectivity are reversed from the PrPCJD accumulation in the salivary gland during the late post-inoculation time-course; that is, infectivity declined as PrPCJD accumulated in this tissue. This strongly suggests that PrPCJD is not itself the infectious agent, if the former is an essential component of the latter. No correlation between the kinetics of these factors in the spleen supports this idea.

Inaccuracy of a quantitative bioassay of the infectious agent may make interpretation of the results difficult in this kind of study. Recently, Race & Ernst (1992) described the lack of a quantitative correlation between PrPCJD and infectivity in the spleen of scrapie-infected mice, but they interpreted the discrepancy to be due to inaccuracy of the infectivity assay, an endpoint titration. They investigated the infectivity titration error by determining the infectivity titres of eight different but equivalent spleen homogenates, and discovered that there appeared to be a 10- to 15-fold difference in comparable samples. It is important to note that the infectious agent can easily aggregate with cellular elements and itself. If aggregation occurs in a serial dilution, an endpoint titration assay may produce a falsely low infectivity titre. In the present study, we measured infectivity by the incubation period method formalized by Prusiner et al. (1980), in which undiluted samples are used, thus minimizing the possibility of aggregation. Indeed, as shown in Table 2, differences in incubation periods of four to six comparable samples appeared to be less than 10%.

Bessen & Marsh (1992) observed differences in properties of sedimentation in sarcosyl and sensitivity to
digestion with proteinase K between PrPs raised by two different strains of the transmissible mink encephalopathy (TME) agent. They concluded that the differences in biochemical properties were due to different post-translational modifications of the two PrP\textsuperscript{TME}. Since we quantified PrP\textsuperscript{CJD} after sedimentation in sarcosyl and treatment with proteinase K, it is possible to argue that some parts of the PrP\textsuperscript{CJD} molecules in salivary gland or spleen might be lost during the process of purification due to differences in post-translational modification among the tissues. Determination of the quantity of PrP\textsuperscript{CJD} in unprocessed original lysates used for the infectivity assay may be needed to clarify further the relationship between PrP\textsuperscript{CJD} accumulation and infectivity titre in the tissues. Meanwhile, the available information indicates that the difference in post-translational modification results in the different mobility of the protein in polyacrylamide gels. As shown in Fig. 2, the mobilities of three PrP\textsuperscript{CJD} species from salivary gland were identical to those from the brain, suggesting that the post-translational process is similar among the tissues. An additional fast migrating band was detected in the spleen tissue. This may have been an alternatively processed species of PrP\textsuperscript{CJD} with the other tissues. It is more likely that the additional fast migrating band reflects the high endogenous proteolytic activity of the spleen tissue.

Although kinetics of infectivity in the salivary gland and spleen were distinct from those of the brain, the time-course and rate of PrP\textsuperscript{CJD} accumulation were similar among the tissues. This indicates that the mechanism of PrP\textsuperscript{CJD} accumulation is not the same as that of the agent replication. It has been proposed that PrP\textsuperscript{Sc} (PrP\textsuperscript{CJD}) itself causes the conversion of PrP\textsuperscript{C} to its protease-resistant form, PrP\textsuperscript{Sc} (PrP\textsuperscript{CJD}), although the nature of the conversion is unknown. When exogenous PrP\textsuperscript{CJD} is introduced into cells, the conversion may occur autonomously regardless of the cell type. On the other hand, replication of the infectious agent seems to be regulated in a tissue-specific manner. This can be explained fully by the idea that the infectious agent is not the same as PrP\textsuperscript{CJD}. The possibility remains that tissue-specific, non-infectious cellular components are involved in either activation or inactivation of the specific infectivity of PrP\textsuperscript{CJD} and that the specific infectivity among tissues may change over time.

Our present findings strongly suggest that PrP\textsuperscript{CJD} is unlikely to be the agent itself, but that PrP\textsuperscript{CJD} accumulation is associated with replication of the infectious agent. Further studies are needed to elucidate the nature of the infectious agent in CJD and scrapie.

We are grateful to Drs J. Tateishi and M. Shina-gawa for providing the reagents. This work was supported in part by a grant from the Ministry of Health and Welfare of Japan.

References


CJD agent in salivary glands


(Received 1 April 1993; Accepted 9 June 1993)